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BAKER & MCKENZIE LLP
Pennzoil Place, South Tower
711 Louisiana, Suite 3400
HOUSTON, TX 77002-2716

EXAMINER	
WALICKA, MALGORZATA A	

ART UNIT	PAPER NUMBER
1652	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Advisory Action Before the Filing of an Appeal Brief	**Application No.** 10/808,717	**Applicant(s)** SAN ET AL.	
	Examiner Malgorzata A. Walicka	**Art Unit** 1652	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 29 March 2007 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

1. ☒ The reply was filed after a final rejection, but prior to or on the same day as filing a Notice of Appeal. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. The reply must be filed within one of the following time periods:
   a) ☒ The period for reply expires 3 months from the mailing date of the final rejection.
   b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

   Examiner Note: If box 1 is checked, check either box (a) or (b). ONLY CHECK BOX (b) WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☐ The proposed amendment(s) filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   (a) ☐ They raise new issues that would require further consideration and/or search (see NOTE below);
   (b) ☐ They raise the issue of new matter (see NOTE below);
   (c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   (d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.

   NOTE: _____. (See 37 CFR 1.116 and 41.33(a)).
4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☒ For purposes of appeal, the proposed amendment(s): a) ☐ will not be entered, or b) ☒ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.
   The status of the claim(s) is (or will be) as follows:
   Claim(s) allowed: _____.
   Claim(s) objected to: _____.
   Claim(s) rejected: 27-33.
   Claim(s) withdrawn from consideration: _____.

AFFIDAVIT OR OTHER EVIDENCE

8. ☒ The affidavit or other evidence filed after a final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
9. ☐ The affidavit or other evidence filed after the date of filing a Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing a good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
10. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

11. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: see the attached.
12. ☐ Note the attached Information Disclosure Statement(s). (PTO/SB/08) Paper No(s). _____
13. ☐ Other: _____.

Advisory Action

Response to Final Action filed March 29, 2007 is acknowledged. Declaration of George N. Bennet under 37 CFR §1.132 is acknowledged, but as indicated in the covering PPO L-303 has not been entered. Pending claims 27-33 are the subject of this action.

Objection to Table 2 are maintained, because the table has not been amended. In description of Fig. 6 the transformant used for production of acetate is named as DH10(pKmAT, pRV380), which according to Table 2 overexpresses only *panK*.

U.S.C. 112, second paragraph

The amendment overcame rejections of claim 27, 29 and 31-33.

35 USC section 112, first paragraph

Written description

Claims 27-33 remain rejected for reasons explained in the final rejection.

Rejections for new matter

The amended Claims 28 and 32-33 are rejected as lacking written description of a transformant having reduced activity of **ackA or pta, which is a new matter, as explained in the final rejection. The amended** Claim 31 is lacking written description of production of any ester (a large genus of esters) by any bacterial cell transformed

and cultivated as in claim 27. This is a new matter. There is nothing in the disclosure which indicates that applicants intended to claim production of any esters. There was no any other esters in the claims as originally filed, and the specification teaches in paragraph [12] "a method of producing isoamyl acetate", and Fig. 10 "illustrates isoamyl concentration in the strain tested".

The disclosure teaches only E. coli cells transformed with panK, ATF2 and pyruvate dehydrogenase in combination and, alternatively, also when ackA and pta of E. coli have reduced activity. These E. coli transformants were grown in presence of pantotenoic acid, a precursor of CoA, and in the presence of isoamyl alcohol, a precursor of isoamyl ester. The presence of alcohol acetyl transferase gene and isoamyl alcohol causes isoamyl acetate production which is not produced in wild type E.coli. The disclosure does not teach a genus of transformants having ackA or pta genes impaired and production of isoamyl acetate does not describe the production of the genus of all esters (claim 31should recite actate esters). E. coli does not identify the genus of bacterial cells that may be used for production of any ester.

Response to Applicant' traversal and arguments regarding new matter

Regarding claim 28 applicants argue (REMARKS, page 6/9, third paragraph) quoting a fragment from paragraph 28 of the specificaiton, "A-CoA may be converted to acetyl phosphate by phosphotransacetylase (PTA), which in turn may be converted to acetate using acetate kinase (ACK)", and continue, "It is equally clear, that if either gene

is deleted or otherwise inactivated, the reaction cannot proceed to completion. Thus the recitation is not a new matter."

The applicants's argument has been fully considered but is found not persuasive for the following reasons. Although it is known in the art that if either gene is deleted or otherwise inactivated the reaction cannot proceed to completion, the transformants that applicants engineered had boths ackA-pta genes affected because the original E. coli had this feature. Applicants have not engineered or used a transformant with reduced activity ackA, and Applicant's have not engineered or used a transformant with reduced pta activity. Thus, claim 28 is rejected for new mater.

Regarding claim 31, on page 4/9, the last paragraph of REMARKS, one reads: "Applicants explained that the demonstration of increased isoamyl acetate was exemplary only. In fact, this is only one of many molecules that can be converted to an ester using the substrate non-specific ATF enzyme. Thus there is no need to include isoamyl alcohol in the medium unless one actually desires to produce isoamyl acetate."

This argument of applicants is found not persuasive because although biochemistry teaches that unspecific ATF my use many alcohols to transfer acetate, applicants were only interested in production of isoamylacete as discussed above. Production of any ester was introduced with the after final amendment. Furthermore, there is nothing in the disclosure and in the art suggesting that any bacterium is capable producing any ester after transfecting with unspecific ATF. It is possible only when the bacterium produces any alcohol, i.e. all alcohols, which is not the case. As the

specification teaches, E. coli does not produce isoamyl alcohol, therefore this very alcohol should be added to the growth medium to produce the isoamyl acetate.

Scope of enablement

Claim 27-33 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the claimed methods wherein E. coli is used as host cell to be transformed with the genes Applicants used, does not reasonably provide enablement for any bacterial host cell and any pandK, pyruvate dehydrogenase and alohol transferase gene to be used in the claimed methods. The reasons for the rejection were explained in the final rejection a previous action.

Response to applicants' argument

In REMARKS, page 5/9, the last paragraph, Applicants state that they do not claim the use of generic genes, but specifically named genes that are well known in the art and encode proteins having the requisite activities (pantotenate kinase, pyruvate dehydrogebnase and alcohol acetyl transferase).

Applicants argument is not found persuasive because although the genes are known in the art and are specifically named by applicants that does not change the fact that there exists extremely large genus of panthotenates kinases, extremely large genus of pyruvate dehydrogenases and extremely large genus of alcohol acetyl transferase. All of these genera comprise all named enzymes from natural sources as well as man made.

35 USC 103

Claims 32-33 are rejected under 35 U.S.C. 103(a) as being unpatentable over San et al. (Metabolic Engineering through Cofactor Manipulation and Its Effects on Metabolic Flux Redistribution in *Escherichia coli*, Metabolic Engineering, February 27, 2002, 4, 182-192, included in the Information Disclosure Statement), in view Vallari D. et al. (Biosynthesis and Degradation Both Contribute to the Regulation of Coenzyme A Content in *E. coli*, J. Bact, 1988, 3961-3966, sent to Applicants with the previous action) and Voet et al. (Biochemistry, second Edition, 1995, John Wiley & Sons, Inc, pp.543-548 enclosed), and Yang et al. (Effect of Inactivation of *nuo* and *ackA-pta* on Redistribution of Metabolic Fluxes in *Escherichia coli*, Biotech. Bioeng. 1999, 65, 291-297, enclosed). The reasons have been explained in the final rejection.

Response to Applicants arguments

I. Applicants position, presented in REMARKS page 8/9, second paragraph, is that even when combined the art does not teach every claimed element and a prima facie case of obviousness is not made. "The Examiner has failed to cite art that teaches the missing elements of having added recombinant PANK and PDH."

This argument is not persuasive because, firstly, rejection under 35 USC 103 is not a rejection under 35 USC 102, which requires the presence in the art of every element claimed. Secondly the examiner has not failed to cite art that teaches having added recombinant PANK and PDH. The related fragment of the rejection states that it

would have been obvious for one having ordinary skills in the art, who would like to produce isoamyl acetate in E. coli, to transform E. coli (bacterial) cell with atf2 as Sun did, and replace an E. coli having overexpressed panK gene by a E. coli into which the pan K gene was introduced to elevate expression of the pantothenate kinase. The motivation is provided by Vallari et al. who teach that panK is the rate-controlling enzyme in CoA biosynthesis in E. coli. The expectation of success was very high because the art at the time of filing taught it was easy to transform E. coli with its own gene as the Applicants did. Thus it was obvious to make E. coli transformed with panK. It, it would also have been obvious for a skilled artisan who would like to produce isoamyl acetate in E. coli to transform E. coli with a gene encoding pyruvate dehydrogenase, because, as expected from the handbook of biochemistry (the copies of related pages were attached) that would increase production of acetyl-CoA which is the substrate for isoamyl acetate; see rejection under 35USC 103, page 14 of the final rejection.

II. Applicants also emphasize that cited references do not teach supplementation with pantothenoic acid, and quote Vadali, et al. (Metab. Eng. 6:133-9 (2004), at page 138),

> "It was found that the intracellular CoA/acetyl CoA could be increased only with the simultaneous overexpression of panthotenate kinase **and supplementation of panthothenic acid**. Since E. coli normally secrets out excess pantothenic acid, it might be logical to assume that the availability of

precursor will not be rate limiting. On the contrary, the **supplementation of panthotenic acid is essential and necessary** for CoA/acetyl-CoA manipulation [emphasis added by applicants in RMARKS]."

In REMARKS applicants conclude: "Thus, at the time of filling, it was unexpected that bacteria would require supplementation with pantothenic acid and, without supplementation increased CoA flux would not be achieved."

Applicants' argument is found not persuasive for the following reasons. The article is based on the very set of data that is in the disclosure of the instant application. The quoted passage originating from the section **Conclusion** is not in accord with the logic of experimentation and results presented in section **Results and Discussion** *Characterization of DH10B (pUC19) and DHN(pRV380) in aerobic shake fasks*, page 135. In this section the authors present results for E. coli transformed with a plasmid comprising **high copy number of E. coli panK genes**. This overexpression, however, did not lead to an increase in the intracellular CoA/acetyl-CoA levels. Although the authors noted that E. coli synthesizes excess amount of pantothenate acid and secrets it to the extracellular medium (right column of page 135, second line of the second paragraph), the authors also concluded (further in the same paragraph), as a skilled artisan would, "With the **overexpression of the panthothenate kinase**, the availability of panthotenic acid might be rate limiting. To test the requirement of concentration of pantothenic acid, a doseage study was performed... The results are shown in Fig.1. The results suggest that ~50 uM of pantothenic acid concentration in the cell culture medium is sufficient **to saturate the enzymatic activity of overexpressed**

pantothenate kinase resulting in highest intracellular acetylCoA elevels [emphasis added by the examiner]."

Applicants' attention is turned to the fact that if E. coli were transformed with one or two additional copies of panK, the supplementation would have been probably not necessary. Also, the concentration of panthotenate in the medium depends on the copy number of panK, and would be different in case of use of a different plasmid comprising different copy numbers than in pRV380. In summary, at the time of filling, it was obvious that bacteria comprising many copies of panK gene would require supplementation with pantothenic acid to increase the CoA flux through the acetyl CoA node. **Thus the supplementation has no feature of novelty.**

Conclusion

The amendment overcame the 112 second paragraph issues, introduced new 112 first paragraph issues and did not overcome the 103 rejection.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Malgorzata A. Walicka whose telephone number is (571) 272-0944. The examiner can normally be reached on Monday-Friday from 10:00 a.m. to 4:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can be reached on (571) 272-0928. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Malgorzata A. Walicka, Ph.D.
Art Unit 1652
Patent Examiner

small corrections after amendment after final
Written description

The examiners. AThey are directed to the increasing CoA flux in any bacterium transformed with a pantothenate kinase gene, pyruvate dehydrogenase gene, and alcohol acetyl transferase gene wherein in addition ackA or pta or both ackA and pta genes have reduced activity and additionally wherein conversion of any alcohol into ester production or particularly isoamyl alcohol into ester is increased in said transformants.

Other remaining rejections for lack of written description

Claims 27-33 are rejected because the origin of genes used for manipulation are not described by the claims. Also, the disclosure does not provide sufficient written description of transformation of any bacterial cell. For their transformation of E. coli applicants used E. coli panK gene and ATF2 gene form yeast, and a not identified pyruvate dehydrogenase gene obtained form yeast. Manipulating metabolism in E. coli cells by transforming them with panK, ATF2 and pyruvate dehydrogenase genes separately or in combination does not provide sufficient written description for transforming any bacterial cells with any species of a genera of panK, ATF and pyruvate dehydrogenase, wherein the genera comprise all natural and man-made genes currently known and to be disclosed. The host cells have their specific requirements as to the expression vector, i.e., plasmid and control expression elements it comprises. Some genes are extremely difficult to be expressed in host different than the cell of origin, and require extensive modifications regarding, for example, their N-termini sequences as well as codons used. Moreover, those skilled in the art realize that metabolic pathways of every cell type are distinct, such that a showing that overexpression of a group of genes in one cell type leads to increased level of CoA may not be true in other cell type.

Furthermore, claims 32-33 are rejected for lack of written description of any bacterial cell that in result of transformation **increased** production of isoamylacete, because the only bacterial cell the Applicants used for transformation, i.e. E. coli, does not produce isoamyl acetate as the wild type. In **E. coli**, the production may not be increased in result of transformation, it **may only start** after transformation and growth

in the presence of isoamyl alcohol. Applicants have not disclosed other host cells that produced isoamyl alcohol production of which has increased after transformation.

All together, Applicants have failed to sufficiently describe the claimed invention in such full, clear, concise and exact terms that a skilled artisan would recognize Applicants were in possession of the claimed invention at the time the application was filed. What Applicant have described is:

1) E. coli transformed with gene (i) is producing more CoA then not transformed, when cultured in the presence of pantothenic acid; see Fig. 5 and 6a,

2) E. coli transformed with (i) and (iii) is able to produce isoamyl acetate, Fig. 6 and 10, in the presence of its substrate isoamly alcohol

3) E. coli transformed with (i), (ii) and (iii) has higher production of isoamylacetate than the one transformed with (i) and (iii).

Applicants also have shown that addition to the culture of transformants of substrate for production of CoA i.e. pantothenic acid increases production of isoamyl acetate in transformants listed under 2) and 3) above; see Fig. 10.

Scope with some small corrections after amendment after final
; see also the above rejection for lack of written description. The specification does not enable any person skilled in the art to which it pertains, or with which it is most

nearly connected, to make and use the invention commensurate in scope with these claims.

The nature and breadth of the claims covers using any bacterial cell for generating a transformant comprising pantothenate kinase gene, pyruvate dehydrogenase gene, and alcohol acetyl transferase genes, wherein said transformant produces more CoA than its nontransformed counterpart, wherein additionally the host cell exhibits reduced activity of ackA, pta or ackA and pta genes, and wherein isoamyl acetate is produced.

While manipulating metabolism of bacterial cell by expressing recombinant enzymes is well developed and the skills of artisans high, not every gene encoding the enzymes listed generically by the claims may be expressed in any bacterial cells. Genetic code usage is specific for the cell to be transformed. The host cells have also specific requirements as to the expression vector, i.e., plasmid and control expression elements it comprises. Some genes for one organism require modification of N-terminus to be exprsssed in some bacteria. Furthermore, providing for E. coli transformed with panK or with panK plus dehydrogenase gene is not a sufficient guidance for the genera of panK and pyruvate dehydrogenase genes and bacterial cells to be used by a skilled artisan to make the invention as claimed. In addition, Applicants' own data suggest that without supplying the growth medium with the pantothenic acid and isoamyl alcohol overexpressing panK, dehydrogenase and ATF alone is not sufficient for an increase in CoA flux. Moreover, those skilled in the art realize that metabolic pathways of cells of every cell type are distinct, such that a showing of overexpression of a group of genes

in one cell type leads to increased level of CoA may not be true in other cell types as well.

Furthermore, Applicants do not enable a cell having reduced activity of ackA, pta or ackA and pta genes, because Applicants just used one E. coli natural mutant (YBS121) having activity of the ACKA and PTA enzymes reduced. The source of the mutant is not stated. Applicants do not teach the structure of E. coli ackA and pta genes and mutation(s) that reduce the activity of the encoded enzymes. Thus, reduction of ACKA and PTA activity is not enabled, imposing on the skilled artisan an experimentation that is undue.

Moreover, those skilled in the art realize that metabolic pathways of every cell type are distinct such that a showing overexpression of a group of genes in one cell type leads to increased the level of CoA may not be true in other cell types as well.

In addition, both three genera of genes and the genus of cells to be transformed, encompass an extremely large number of species and there is no teaching by Applicants which gene, expression vector and cell to chose to get a successful combination.

In summary, a skilled artisan, who would like to make and use the claimed invention is forced to experimentation that has a low probability of success without additional teaching as to bacterial cells and identifying genes that are to be used in the invention. In conclusion, to make and use the broadly claimed invention requires experimentation that is improperly extensive and undue.

REBECCA E. PROUTY
PRIMARY EXAMINER
GROUP 1600
1600